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*ON THE DETERMINATION OF THE GEOCENTRIC DISTANCE IN THE LAPLACE-LEUSCHNER DIRECT METHOD FOR PARABOLIC ORBITS**,***

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In the computation of a parabolic orbit by the direct Laplace-Leuschner method (as in the classical Olbers method) the geocentric distance of the unknown body is obtained from the equation of the sixth degree

$$(z - p)^2 + q^2 = \frac{h}{\sqrt{(z - c)^2 + s^2}} \qquad (1)$$

where z denotes the ratio of the unknown distance to the distance Earth–Sun at the instant of the middle observation, and the parameters p, q^2, h, $c = \cos\psi$, $s = \sin\psi$ have numerical values derived from the observations and the ephemerides. ψ as usual is the elongation of the comet.

Among the different graphical methods proposed for solving this equation let us mention those of Mr. Banachiewicz[1] followed by a discussion on the number of real positive roots and of Mr. B. Strömgren.[2] In this short note it will be shown how Leuschner's graphical treatment of the fundamental equation may be generalized by means of the change of variables

$$xs = z - c, \quad x_0 s = p - c, \; y_0 = q^2 s/h, \quad k = s^3/h, \quad s \neq 0. \qquad (2)$$

This substitution transforms (1) into

$$f(x) = (x^2 + 1)^{-1/2} - k(x - x_0)^2 - y_0 = 0, \qquad (3)$$

which is readily solved by a nomograph. It is sufficient to construct on a transparent sheet of paper the system of parabolas $y = kx^2$, and on another sheet the curve (C) $y = (x^2 + 1)^{-1/2}$, then to place the parabolas in proper relative position with respect to (C) and to read off the abscissae of the points of intersection of the particular parabola k with the quartic curve (C). The approximate values of the roots thus obtained will be corrected by Newton's method, or by interpolation, using for this purpose

a table of values of the function $(x^2+1)^{-1/2}$ and its derivative $-x(x^2+1)^{-3/2}$. Since a change of scale transforms any one of the parabolas into another one of the same family, one may vary if necessary the modulus of the scales in a convenient ratio, an operation which will require the plotting of only one new curve (C).

In the doubtful cases where the graphical method fails the form of equation (3) allows an easy computation of the necessary and sufficient conditions for the existence of three positive roots in z, i.e.,

$$f(x_1') > 0, \quad f(x_2') < 0, \quad f(x_3') > 0, \quad -\cot\psi < x_2',$$

where the three roots x_i' of the derivative are rapidly calculated by finding the intersections of the straight line $y = 2k(x_0 - x)$ with the curve $y = x(x^2+1)^{-3/2}$, or if necessary by the table.

The consideration of the limiting case where the parabola k is osculatory to the curve (C) at point $[t, (t^2+1)^{-1/2}]$ shows that equation (3) will have 4 real roots only if the point (x_0, y_0) lies within the region bounded by the axis of x and a unicursal curve (C') $x_0 = \dfrac{3t^3}{2t^2-1}$, $y_0 = (t^2+1)^{-1/2}\left[1 - \dfrac{1}{2}\dfrac{t^2}{2t^2-1}\right]$, which has a cusp at point $t = \sqrt{\dfrac{3}{2}}$ and is made up of two branches respectively asymptotic to the curve (C) and to the straight line $18y + 2x\sqrt{3} - 7\sqrt{6} = 0$. Besides it is necessary that the parabola k should lie between the 2 parabolas passing through (x_0, y_0) and tangent to (C). Among all the systems of values (x_0, y_0, k) yielding 4 real intersections the only acceptable are those for which the equation

$$\phi(u) = (u^2+1)^{-1/2} - \lambda k(u - x_0)^2 - \lambda y_0 = 0$$

has two real roots falling between the first and the second root of $f(x) = 0$. The auxiliary equation $\phi(u) = 0$ is obtained by elimination of p, q^2, h between the system of equations (2) and the relation $p^2 + q^2 = h\lambda^{-1}$, where the factor λ^{-1} has been introduced in order to take care of the eccentricity of the Earth's orbit. Its numerical value oscillates between 0.4916 and 0.5084.

The determination of the necessary conditions for multiple solutions in the astronomical problem leads to the study of the variations of the parameters ψ, h and p when (x_0, y_0, k) take all the possible values within the domain defined above. Two cases are to be considered according to the sign of x_0.

For a given negative x_0 it can be shown that the extrema of the parameters ψ, h, p take place when y_0 has its smallest possible value; hence by moving point (x_0, y_0) along the lower branch of the limiting curve (C') we shall find the first group of conditions

$$0° \leqq \psi \leqq 18° 8'; \quad 0 < h \leqq 0.64834; \quad -0.5741 \leqq p \leqq 0.2093,$$

corresponding to $x_0 = -2\sqrt{6}$, $y_0 = 0$.

If on the other hand x_0 is positive the extrema occur on the upper branch of (C') and in letting x_0 approach infinity we obtain the second group

$$0° \leqq \psi \leqq 180°; \quad h \geqq 106.83; \quad p \geqq 6.1173.$$

The identification of the two systems of parameters enables us to find at once Mr. Banachiewicz's results.

One will therefore understand why so few comets with 3 solutions have been encountered; the only observed ones so far being 1882 b ($\psi = 6° 19'$, $h = 0.2615$, $p = -0.2171$) and 1910 a ($\psi = 6° 33'$, $h = 0.0838$, $p = 0.1755$) both of the first group. The application of the preceding method to a difficult case has led to the discovery of a concrete example belonging to the second group, namely, the case of the Schwassmann comet, 1927 j ($\psi = 147° 26'$, $h = 210.6$, $p = 10.18$) with the three geocentric distances 2.560, 3.302, 13.597. But on account of the range of solutions due to the uncertainty of the observations it might belong to the class of comets with a unique solution.

As for the case $s = 0$, $c = \pm 1$, it is disposed of directly by solving the simplified equation

$$\pm m[(m-1)^2 + \beta] - \alpha = 0,$$

where $m = \dfrac{x}{x_0}$, $x_0 = p \pm 1$, $\alpha = \dfrac{h}{x_0^3}$, $\beta = \dfrac{q^2}{x_0^2}$ the lower sign being used for finding the 4th root in x which always has the opposite sign of x_0.

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[1] *Compt. rend.*, tome **161**, 122 (1915).

[2] *Publ. Obs. Copenhagen*, No. 66, 24 (1929).

[3] *Publ. Lick Obs.*, **7**, 282, ff. (1913).

THE RATE AND EXTENT OF ANAEROBIC DECOMPOSITION OF SULFITE WASTE LIQUOR BY BACTERIA OF SEA WATER MUD*

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I. *Introduction.*—Sulfite waste liquor is the liquid product resulting from the manufacture of paper pulp by the acid-sulfite process. During the process the hemicelluloses of the wood are hydrolyzed into soluble sugars; the lignin forms a soluble complex known under the name of "calcium lignosulfonate" and the cellulose remains as pulp. The liquor then contains in solution in a somewhat modified form all the constituents of the original wood with the exception of its cellulose content.

This waste liquor (s. w. l.) is discharged into natural water courses. The question of what then happens to it has been very little investigated, and there is no record as to the extent of its decomposition under natural conditions. Benson[2] studying the pollution of Oakland Bay, in Puget Sound, on which a pulp mill is located, found that there is no accumulation of organic matter in the Bay and concluded that the s. w. l. is evidently being broken down by the action of microörganisms naturally occurring in the sea water.

Since the principal organic constituent of the s. w. l. is lignin, the destruction of the liquor by the natural biological processes should bear a close resemblance to the decomposition of lignous material during the decay of wood. The exact chemical changes taking place in decaying wood are influenced by the chemical composition of the plant constituents[17] and are subject to environmental conditions, such as the hydrogen-ion concentration, the moisture supply and aeration, all of which govern the nature of the microörganisms taking an active part in the decomposition processes. Various fungi, molds, actinomyces, aerobic and anaerobic bacteria may predominate, depending on the conditions mentioned above.

The concurrence of opinion at the present is that the lignin of plant substance is the most resistant part of the plant material.[4,12,14,15,16] It is decomposed to a certain extent together with cellulose and pentosans under aerobic conditions, the relative decomposition becoming much slower under anaerobic conditions. Only in the case of white rot is the lignin in the wood preferentially attacked and depleted by the process of oxidation,[18] which according to Campbell[5] occurs under the influence of an oxidase excreted by the fungus. Pure lignin prepared by the known chemical means is still more resistant to decomposition by microörganisms;[11,14] this is very likely due to the changes in its composition under the treatment by strong alkali, hydrochloric and sulfuric acids in the processes of its isolation.[8]

The lignin in the s. w. l. was subjected to even more drastic chemical treatment.

II. *Object of Experimental Study.*—The object of this investigation was to study the influence of temperature and dilution of the s. w. l. by sea water on the rate and extent of its decomposition and stabilization under strictly anaerobic conditions by the bacteria of the sea water mud, and, if possible, determine the nature of the residual products produced during the fermentation.

III. *Selection of Temperatures.*—The temperatures selected approximated as nearly as possible those of natural conditions, and for which constant incubating facilities were available.

The fermentation vessels were incubated at three different temperatures:

9°C., the average temperature of the Puget Sound waters;

21°C., the temperature commonly reached by the waters retained on beaches at low tide during the hot period of the year;

36°C., maximum summer temperature of sea mud exposed at low tide.

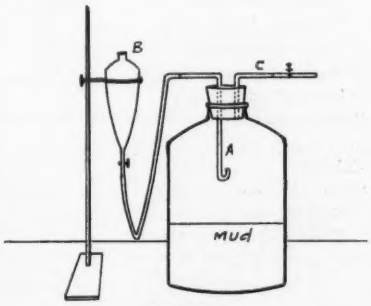

FIGURE 1

IV. *Fermentation Vessels.*—The choice of the size of the vessels was governed by the approximate rate of gas evolution determined in the preliminary experiments. For 9°C. 10-liter bottles were selected while for the other two temperatures one-gallon bottles were used. Bottles used for gas studies were provided, after R. J. Allgeier,[1] with a tube, *A*, (Fig. 1) which passed through a tightly fitting rubber stopper and extended about a third of the way into the bottle. It was connected by means of a rubber tube to a separatory funnel, *B*, supported on a ring stand. A capillary tube, *C*, passing only to the bottom of the stopper was provided for the removal of gas samples. As the gas formed, the liquid was forced from the bottle through the tube, *A*, into the funnel, *B*. By measuring the amount of liquid in the funnel, *B*, the volume of gas produced was ascertained. Samples of gas were taken when at least 75–100 cc. of gas were produced and all gas in excess of that used for analysis was removed at the time samples were taken. In order that the gas evolution studies might not be disturbed, duplicate sets of bottles were used for bacterial counts.

V. *Marine Mud.*—The mud was collected at low tide from Huston oyster beds, located at Mud Bay, seven miles south of Olympia, Washington, on February 11, 1933. The collected mud was thoroughly mixed in

a large vat and weighed out into the bottles: 2.00 kgrs. into the one-gallon bottles and 4.50 kgrs. into the 10-liter ones. This mud contained 38.5% moisture. On the oven dry basis the mud had 21.1% of carbonates, as $CaCO_3$, 0.3% of carbon other than in carbonates (determined by combustion) and about 35% of sand.

VI. *Sulfite Waste Liquor.*—The sulfite liquor, obtained from a pulp mill at Shelton, Washington, through the courtesy of Mr. R. N. Pollock, was a "strong blow liquor" taken from a digester just before a blowing period.

TABLE 1

ANALYSIS OF THE LIQUOR USED

Density at 24°C.		1.045
Free acidity by titration		0.294 Normal
Total solids, oven dried at 105°C.		11.90 grms. per 100 cc. of liquor
Carbon in the solids	45.0%	
Hydrogen in the solids	5.1%	
Residue on ignition	9.0%	
Reducing sugars, as glucose (average on liquors)	2.6%	
Permanganate consumed (in 1:4000 dilution)		113.4 mgrs. of oxygen per 1 cc. of the liquid
Calcium		4.78 grms. per liter
Sulfur (S) total, per liter		11.31 grms.
Free SO_2 per liter		7.31 grms.
Combined SO_2 per liter		15.30 grms.

TABLE 2

DILUTIONS OF THE SULFITE LIQUOR USED

DILUTION	TEMPERATURES OF INCUBATION	CC. OF LIQUOR PER GALLON BOTTLE	DILUTION % BY VOLUME OF SULFITE LIQUOR TO TOTAL LIQUIDS	GRMS. OF SOLIDS IN THE LIQUOR PER 100 GRMS. OF MUD	GRMS. OF ORGANIC CARBON PRESENT PER GALLON BOTTLE
B	9, 21	45	1.36	0.38	2.41
C	9, 21, 36	175	5.30	1.49	9.36
D	9, 21, 36	540	16.4	4.58	28.95
E	21, 36	870	26.4	7.39	46.60

Acidity of the liquor was neutralized by treatments first with $CaCO_3$ and then with $Ca(OH)_2$. The precipitated calcium sulfite and that part of the organic matter which separated were kept in suspension by agitation while the liquor was measured into different bottles, which already contained the mud. The bottles were then filled to the neck with sea water taken from the tanks of the oceanographic laboratory, stoppered, the contents mixed and incubated.

VII. *Dilutions Studied.*—Four concentrations: *B*, *C*, *D*, *E*, and a blank *A*, were used. They are given in table 2.

VIII. *Analytical Procedure.*—The gas produced was analyzed by means of a Williams modification of the Orsat apparatus for CO_2, H_2, CH_4 and for H_2S by titration of a separate sample with standard iodine solution. At the end of the fermentation period the dissolved gases were analyzed using Greenberg's adaption for sea water of the Van Slyke method.[6] The hydrogen-ion concentration was determined periodically by means of La Motte colorimetric pH sets. After the 100th, 200th and 300th days samples of the liquor were taken and the following determinations made: total solids, oxygen consumed and total sulfur.

For the total solids determination 10 cc. samples were evaporated to dryness and the residue dried in an oven at 105°C. for 20 hours. The total solids were analyzed for carbon and hydrogen by the micro-combus-

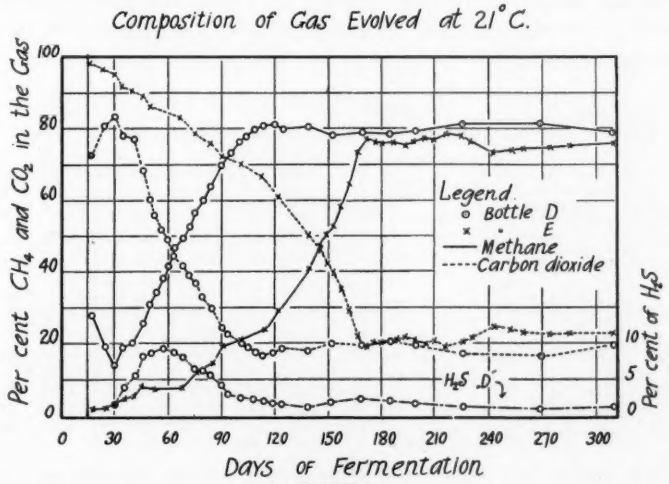

PLATE 1

tion method of Pregel.[10] Oxygen consumed test was made by the Benson modification[3] of the A.P.H.A. method. Sulfur was determined by the permanganate method of Pollock and Partansky.[9] At the end of the fermentation period dissolved gases (primarily CO_2 and H_2S) and reducing sugars (reported as glucose) were determined. The fermenting solutions were practically distilled—the first half with the solution alkaline and the rest to dryness after having been made acid with H_3PO_4. The first fraction was tested in the usual way for ethyl and methyl alcohols, acetone and phenol groups, and the second fraction was titrated for organic acids, computed as acetic acid.

IX. *Bacteriological Studies.*—During the course of the experiment periodical bacterial counts were made using an ordinary peptone-glucose

agar media made up with sea water. Both aerobic and anaerobic plates were made and were all incubated at 21°C.

A number of bacteria taken from bottle "*E*" incubated at 21°C. were isolated and their characteristics in pure culture studied in detail; this will be a subject of a later communication.

X. *Discussion of Experimental Results.*—1. *Fermentation gases.* Analysis of the gas evolved showed that it consisted of carbon dioxide, methane, hydrogen sulfide and nitrogen. However, nitrogen was found in the evolved gas only at the beginning of fermentation; its presence was due to the sweeping out of the dissolved air that was originally present in the water and in the mud. Results have, therefore, been computed on a nitrogen free basis.

Hydrogen was never found in amounts exceeding an experimental error of 0.5% of the gas determinations, showing that the butyric type of fermentation was absent.

TABLE 3

EVOLVED GASES IN CC. PER LITER OF SOLUTION

		IN THE GASEOUS PHASE						IN SOLUTION		TOTAL GAS PRODUCED				
		100 DAYS	200 DAYS	AFTER 310 DAYS				AFTER 310 DAYS AND						
	BOTTLE NUMBER	TOTAL GAS	TOTAL GAS	CO_2	CH_4	H_2S	TOTAL GAS	FUNNEL LOSSES CO_2	H_2S	CO_2	CH_4	H_2S	TOTAL CO_2 & CH_4	PERCENTAGE OF CARBON GASIFIED
9°	C	2	14	12	30	3	45	747	297	759	30	300	789	17.7
	D	13	85	119	43	15	177	1391	471	1510	43	486	1553	11.7
21°	B	...	...	...	...	...	...	342	...	342	...	...	342	25.1
	C	119	264	95	187	19	301	1094	153	1189	187	172	1376	26.0
	D	1628	2415	872	1512	106	2490	1802	171	2674	1512	277	4186	26.2
	E	749	3055	1530	2390	168	4088	2152	266	3682	2390	434	6072	23.5
36°	C	606	625	257	336	32	625	825	106	1082	336	138	1418	26.8
	D	3547	3630	1516	2022	92	3630	1471	149	2987	2022	241	5009	30.4
	E	4270	4885	2023	2738	220	4981	1674	291	3699	2738	511	6437	25.1

In all cultures the percentage of hydrogen sulfide rapidly rose to a maximum of 8 to 11 per cent of the gas volume, and then immediately proceeded to recede gradually. It came, we believe, from the sulfites of the s. w. l. rather than from the sulfates of the sea water or the lignosulfonic acids, for all sulfur reducing bacteria isolated and studied were incapable of reducing sulfates but attacked sulfites readily.

At the very beginning of fermentation, due to dissolved oxygen, partially aerobic conditions prevailed and the carbon dioxide constituted up to 100% of the gas. Its percentage, however, decreased gradually, reaching a constant value of about 20%. Methane, absent at the very beginning in all cases, gradually rose to a constant value of 77 to 80% of the gas. The time required for CO_2 and CH_4 to reach their respective constant values depended upon the dilution of the liquor and the temperature of incubation and corresponded to the time when the gasification rate reached

its maximum; dilution "*D*" at 36°C., for example, reaching the equilibrium value in 40 days.

The gas data are given in table 3. It is seen that the total amount of gas produced in 310 days, after correction for dissolved CO_2 has been made, is practically the same as for corresponding dilutions incubated at 21° and 36°. The change in the composition of the evolved gases with time for "*D*" and "*E*" incubated at 21°C. is shown in plate 1. The same graph could be made to represent dilutions "*D*" and "*E*" incubated at 36° if the time coördinate is changed from 310 days to 130 days.

The rate of gas evolution was the fastest at 36°C.; it commenced in five days and at the height of fermentation over 154 cc. were evolved per day per liter of solution. At 21°C. the first sample of gas large enough for analysis was collected after 17 days, and at 9°C. after 63 days.

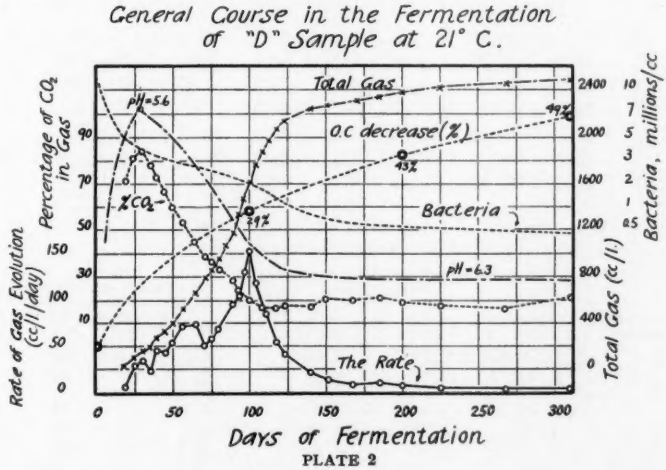

PLATE 2

2. *Hydrogen-Ion Concentration.*—The pH of the fermenting solutions was found, in general, to depend on two factors: concentration of the sulfite liquor, and dissolved CO_2 (function of its partial pressure in the gas phase and temperature). As soon as fermentation set in, there was a sudden drop of pH due to the formation of acids and of dissolved CO_2. The rise in pH followed a drop in CO_2 production. This is shown in the case of a typical bottle "*D*" at 21°C.—Plate 2, where the two curves appear parallel.

3. *Carbon Balance.*—Due to the complexity of the changes taking place in the organic matter of the s. w. l. during fermentation, a simple carbon balance was used in following the disappearance of the organic matter from the solution. From the ultimate analysis of the liquor and the

amount of it used we know the carbon input in each bottle. After completion of the fermentation the carbon balance was as follows: organic carbon left in solution in the form of dissolved solids, carbon removed from the solution in the form of a gas; dissolved carbon dioxide and that lost from solution in separatory funnels; and carbon unaccounted for. The carbon balance is given in table 4.

TABLE 4

TOTAL SOLIDS AND CARBON BALANCE

		TOTAL SOLIDS, GRAMS PER LITER DRIED AT 105° C.		CARBON BALANCE IN GRAMS PER LITER AFTER 310 DAYS						
						% CARBON DISTRIBUTION				
	BOTTLE NO.	ORIGINAL	AFTER 310 DAYS	ORIGINAL CARBON GRMS. PER LITER	GRAMS CARBON FOUND IN SOLIDS	IN SOLIDS	IN GAS	CO_2 IN SOLUTION AND CORRECTION	TOTAL GASIFIED	UNACCOUNTED FOR
9°	B	32.45	32.77	0.60	...	...	0	...	...	...
	C	35.40	33.56	2.41	1.96	81.3	0.9	16.8	17.7	1.0
	D	43.28	40.36	7.23	6.28	86.8	1.22	10.5	11.7	1.5
21°	B	32.68	32.00	0.73	0.40	55	0	25	25	20
	C	36.10	33.10	2.84	1.67	59.2	5.4	20.6	26.0	14.8
	D	45.80	30.74	8.77	5.08	57.9	14.5	11.7	26.2	15.9
	E	54.50	40.14	14.13	8.14	57.6	15.0	8.5	23.5	18.9
36°	C	36.10	32.51	2.84	1.59	56.0	11.2	15.6	26.8	17.2
	D	45.80	36.21	8.77	5.22	59.6	21.6	9.0	30.6	9.8
	E	54.50	39.67	14.13	8.50	60.1	18.6	6.5	25.1	14.8

TABLE 5

OXYGEN CONSUMED

		GRAMS OF OXYGEN CONSUMED PER LITER OF SOLUTION			PERCENTAGE OF THE ORIGINAL (0 DAYS—100%)		
	BOTTLE NUMBER	AT THE BEGINNING	AFTER 100 DAYS	AFTER 200 DAYS	AFTER 310 DAYS	100 DAYS	200 DAYS	310 DAYS
9°	C	5.08	3.68	3.45	3.89	72	68	77
	D	15.42	14.4	10.62	10.20	94	69	66
21°	B	1.54	...	0.83	0.75	..	54	49
	C	5.98	3.78	3.31	3.06	63	55	51
	D	18.59	13.12	10.62	9.40	71	57	51
	E	29.75	18.7	16.43	14.63	63	55	49
36°	C	5.98	3.61	3.41	3.07	60	57	51
	D	18.59	13.5	11.68	10.3	73	63	53
	E	29.75	18.72	16.20	14.7	63	54	49

4. *Oxygen Consumed.*—The oxygen consumed values are given in table 5. It is noted that these values steadily decreased in all bottles, reaching after 310 days in the cultures incubated at 21° and 36°C. a uniform and constant value of about 50% of the original.

5. *Estimation of Sugars.*—The reducing sugars determinations reported as glucose in table 6 include all substances that reduce cupric copper in alkaline solution. These values are about 15% of the original and show that at least 85% or more of the "sugars" were destroyed. The reducing sugars, calculated as glucose, are present in the liquor in the amount of

2.6%; the glucose carbon, however, constitutes only 19% of the total carbon present in solution. With the average gasification of 25% of the carbon input we may assume that certain small amounts of other substances besides the reducing sugars were attacked and gasified by bacteria.

6. *Distillation Tests.*—It is generally agreed that in the fermentation of organic matter acids are intermediate products primary to gas formation,[13,14] especially under anaerobic conditions. Only a very small amount of organic acids and their salts remained in the cultures, as was shown (table 7) by titration of the distillation products of solutions in which, after 310 days, fermentation had practically ceased, while in the 9° cul-

TABLE 6

REDUCING SUGARS AS GLUCOSE

(Data on per liter basis)

BOTTLE NUMBER		GRMS. ORIGINALLY PRESENT PER LITER	GRMS. FOUND AT END OF FERMENTATION	GRMS. APPARENTLY DESTROYED	% GLUCOSE APPARENTLY DESTROYED
9°	C	1.16	0.365	0.795	68.5
	D	3.48	1.036	2.44	70.3
21°	C	1.37	0.37	1.00	72.8
	D	4.23	0.57	3.66	86.3
	E	6.80	0.70	6.10	89.6
36°	C	1.37	0.33	1.04	75.6
	D	4.23	0.61	3.62	85.5
	E	6.80	0.95	5.85	86.0

TABLE 7

ORGANIC ACIDS IN THE LIQUID AFTER 310 DAYS

BOTTLE NUMBER		COMPUTED AS ACETIC ACID IN GRMS. PER LITER
9°	C	0.174
	D	2.310
21°	D	0.264
	E	0.462
36°	D	0.243
	E	0.214

tures in which fermentation was still active, concentration of the organic acid radicals was roughly ten times that of the former.

XI. *Oxidation–Reduction Products Balance.*—The only oxidation product of fermentation was carbon dioxide. There were three reduction products: hydrogen sulfide, acids and methane. Reduction of sulfites and formation of hydrogen sulfide and carbon dioxide were the first reactions taking place. This is evidenced by a high percentage of carbon dioxide and by a rapid rise of the percentage of hydrogen sulfide in the gases at the beginning of fermentation and a correspondingly low percentage of methane. As soon as the supply of sulfites began to be exhausted the

percentage of methane in the gas rapidly increased; the H_2S in the gas at this stage coming primarily from the sulfites dissolved in the liquid, which were produced at the beginning. The amount of intermediate products—acids—present in any particular bottle at any one time varied, depending on the relative rates of the two reactions:

$$\text{Organic matter} + H_2O \longrightarrow CO_2 + \text{organic acid} \quad (1)$$

$$\text{Organic acid} + H_2O \longrightarrow CO_2 + CH_4. \quad (2)$$

The second reaction was not always able to keep up with the first, causing accumulation of acids. Such periodical accumulation of acids was also noted by Symons.[13] This fact accounts for the fluctuation in the rate of gas evolution and its composition, especially noted in the case of bottles containing s.w.l. in higher concentrations and during the early fermentation period. The 9°C. cultures at the time of the termination of the experiment were still at the stage of CO_2-H_2S-acids production.

Summary.—1. Anaerobic fermentation of the sulfite waste liquor by sea mud bacteria was studied in four dilutions and at three different incubation temperatures.

2. At the end of 310 days fermentation at 36°C. was complete, and at 21°C. it had practically ceased, but at 9°C. the cultures were still fermenting.

3. As measured by all tests used, the temperature of incubation and dilution of the liquor governed the rate of fermentation but did not appreciably affect the extent of the decomposition.

4. Sugars of the liquor were destroyed to the extent of at least 85%.

5. The average extent of gasification—25% of the carbon input—was somewhat larger than could be accounted for from the decomposition of sugars alone.

6. The oxidation product of fermentation was carbon dioxide; the reduction products were methane and hydrogen sulfide. The latter was preferentially formed when sulfites were available.

7. The oxygen consumed at the end of 310 days at both high temperatures was equal to about 50% of the original, while the 9°C. cultures were 70% of the original.

8. The resultant product of anaerobic fermentation of s.w.l. was a stable material of a faint odor and after aeration did not cause any change in the oxygen consumed.

9. The evolved gases on the average had a heating value of 649 B.t.u. per cubic foot. Based on the yield obtained at 36°C. the heating value of the gases evolved per ton of pulp amounted to 1,430,000 B.t.u. or 5610 horse-power hours.

* Acknowledgment is made to the NATIONAL RESEARCH COUNCIL for the Grant-in-aid whereby this study was made possible.

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NOTE ON THE LOCATION OF THE CRITICAL POINTS OF HARMONIC FUNCTIONS

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It is the object of this note to state two theorems relative to the location of the critical points of harmonic functions in two dimensions, under certain simple restrictions on given equipotential (level) curves. We leave most of the details of the proof to the reader, and merely mention here that a natural proof can be given (i) by approximation of the harmonic functions involved by the logarithms of the absolute values of certain rational functions whose numerator and denominator have the same degree[1] and (ii) application of known results on the location of the roots of the jacobian of two binary forms and of the derivative of a rational function.[2] This method of proof has recently been used[3] in the study of equipotential curves of Green's function, and thereby yields certain results analogous to—in fact, limiting cases of—the present results, and which indeed can be proved by the use of the present results.

In the following theorems we assume for definiteness that the given harmonic function takes the values zero and unity on given sets of curves; it is obvious that any other pair of distinct numbers can be used instead.

THEOREM I. *Let a region R of the extended plane be bounded by two sets of Jordan curves $C_1', C_2', \ldots, C_\mu', C_1'', C_2'', \ldots, C_\nu''$, of which no two curves intersect. Let the function $u(x, y)$ be harmonic at every interior point of R, continuous in the corresponding closed region. Let $u(x, y)$ have the value zero at every point of the C_j' and the value unity at every point of the C_k''. Let all the curves C_j' lie in a circular region* (i.e., *closed interior or exterior of a circle, or half-plane*) *C' and all the curves C_k'' lie in a circular region C'' which has no point in common with C'. Then all the critical points of $u(x, y)$ in R lie in C' and C''; these two circular regions contain respectively $\mu - 1$ and $\nu - 1$ such critical points, each point being counted according to its multiplicity. If $P:(x_0, y_0)$ is a point of R exterior to C' and C'', then there exists a circle normal to the curve $u(x, y) = u(x_0, y_0)$ at P which cuts both C' and C''.*

This last sentence can be otherwise expressed. Transform the configuration by inversion in a circle so that P is an internal center of similitude for C' and C''. Then the line normal to the curve $u(x, y) = u(x_0, y_0)$ at P cuts both C' and C''.

In the study of the precise number of critical points of $u(x, y)$ interior to C' and C'', some such reasoning as the following can conveniently be used. This reasoning is based on the use of continuity, which was employed in a particular case for the roots of polynomials by Bôcher. Let C' and C'' be finite, assume for definiteness $\mu \geqq \nu$, and consider the function

$$f(z) = \frac{(z - \alpha_1)(z - \alpha_2) \ldots (z - \alpha_\mu)}{(z - \beta_1)(z - \beta_2) \ldots (z - \beta_{\nu-1})(z - \beta_\nu)^{\mu-\nu+1}},$$

where the point α_k lies interior to C_k' and the point β_k lies interior to C_k''. The finite roots of $f'(z)$ lie in C' and C'', and those circular regions contain respectively $\mu - 1$ and $\nu - 1$ roots of $f'(z)$ distinct from the α_k and β_k. The function $v(x, y) = \log |f(z)|$ is a harmonic function of x and y. If values m and M are suitably chosen, the locus Γ': $v(x, y) = m$ consists of μ non-intersecting Jordan curves respectively interior to the C_k', and the locus Γ'':$v(x, y) = M$ consists of ν non-intersecting Jordan curves respectively interior to the C_k''. The function

$$V(x,y) = \frac{v(x,y) - m}{M - m}$$

satisfies the conditions of Theorem I, where the region considered is now R' (bounded by Γ' and Γ''), so $V(x,y)$ has no critical points in R' or R except in C' and C''. Moreover, the number of critical points of $V(x, y)$ interior to C' and in R' is precisely $\mu - 1$; the number of critical points of $V(x, y)$ interior to C'' and in R' is precisely $\nu - 1$.

Let the loci Γ' and Γ'' be now varied continuously without changing their topological characters, be never allowed to leave C' and C'' respectively, and approach respectively the C_k' and C_k''. We denote by $U(x, y)$ the

variable function which takes on the values zero and unity on the variable Γ' and Γ'' respectively, and is harmonic in the region bounded by Γ' and Γ''. Then (by Lebesgue's theory of variable harmonic functions) the function $U(x, y)$ approaches $u(x, y)$ uniformly in R; hence the critical points of $U(x, y)$ in R vary continuously, never lie outside of C' and C'', and approach the critical points of $u(x, y)$ in R. Therefore the critical points of $u(x, y)$ in C' and C'' are in number as stated in the theorem. Each critical point is to be counted according to its multiplicity.

It is of course true that whenever a function $u(x, y)$ is harmonic in a region R, continuous in the corresponding closed region and takes on the values unity and zero, respectively, on μ and ν components of the boundary of R (having a totality of $\mu + \nu$ components), then the function $u(x, y)$ has precisely $\mu + \nu - 2$ critical points interior to R.

THEOREM II. *Let a region R of the extended plane be bounded by three sets of Jordan curves $C_1', C_2', \ldots, C_\mu'$; $C_1'', C_2'', \ldots, C_\nu''$; $C_1''', C_2''', \ldots, C_\nu'''$, of which no two curves intersect. Let the function $u(x, y)$ be harmonic at every interior point of R, continuous in the corresponding closed region. Let $u(x, y)$ have the value unity at every point of the C_j' and the value zero at every point of the C_k'' and C_k'''. Let all the curves C_j', C_k'', C_k''' lie respectively in circular regions C', C'', C''' no two of which have a common point. Let C_0 denote the locus of the point z_4 defined by the constant cross-ratio*

$$(z_1, z_2, z_3, z_4) \equiv \frac{(z_1 - z_2)(z_3 - z_4)}{(z_2 - z_3)(z_4 - z_1)} = \frac{1}{2}$$

when z_1, z_2, z_3 have C', C'', C''' as their respective loci. Suppose C_0 is not the whole plane; then C_0 is known to be a circular region.

1. *If the entire configuration is symmetric in some point O exterior to C', then all critical points of $u(x, y)$ lie in the regions C', C'', C''', C_0. If in addition these four regions are mutually exclusive, then C', C'', C''' contain respectively $\mu - 1, \nu - 1, \nu - 1$ critical points, and the only other critical point of $u(x, y)$ is at O.*

2. *If the entire configuration is symmetric in some circle C, and if C'' and C''' are mutually inverse in C, then all critical points of $u(x, y)$ lie in the regions C', C'', C''', C_0. If in addition these four regions are mutually exclusive, then C', C'', C''' contain respectively $\mu - 1, \nu - 1, \nu - 1$ critical points, and the only other critical point lies in C_0 on C.*

In both 1 and 2, if C' has no point in common with C'', C''' or C_0, then C' contains precisely $\mu - 1$ critical points of $u(x, y)$.

The conclusion of Theorem II persists even if C'' and C''' intersect and even if some of the curves C_k'' and C_j''' lie wholly or partly interior to both C'' and C''', provided no curve is enumerated both among the C_k'' and the C_j''', and provided the other parts of the hypothesis are satisfied.

Theorems I and II extend immediately (a) to the case where the region R is bounded by mutually exclusive closed point sets C_k', C_k'', C_k''' not necessarily Jordan curves provided the function $u(x, y)$ with the required properties exists and (b) to the case where these mutually exclusive closed point sets C_k', C_k'', C_k''' are infinite in number. Formal proofs of these generalizations are left to the reader, and are conveniently based on Theorems I and II as stated, where the Jordan curves C_k', C_k'', C_k''' of the theorems as stated are taken as level (niveau) curves of the given function suitably chosen near the given point sets C_k', C_k'', C_k'''.

A large number of other results similar to Theorems I and II (in the original or in the generalized form) are easily proved by the same method. We still suppose the function $u(x, y)$ harmonic in a region R, continuous in the corresponding closed region, and to take on the value zero or unity on each component of that boundary. Components $C_k^{(j)}$ of the boundary of R are required to lie in circular regions $C^{(j)}$, and the critical points of the harmonic function $u(x, y)$ are then shown to lie in these circular regions $C^{(j)}$ and in a set of other regions R_ν which are bounded by an algebraic curve whose equation can be given;[4] this curve may consist of one or more circles, as in Theorem II. The equation of the boundary of the R_ν ordinarily involves the value of the integral of the normal derivative of $u(x, y)$ taken over an analytic Jordan curve in R which separates the components $C_k^{(j)}$ for fixed j from the other components of the boundary of R; in certain symmetrical cases (such as Theorems I and II) this integral of the normal derivative can be eliminated.

[1] This is an extension of the method of Hilbert. See Walsh, J. L., *Paris Compt. Rend.*, **198**, 1377–1378 (1934).

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CORRELATION IN PLANARIAN REGENERATION

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The data at present available from the field of experimental embryology and from the experimentation concerned with regeneration and regulation in the adult organism lead us to conclude that the animal which we call typical is not the maximum expression of all of its inherent formative potentialities. The facts necessitate the postulation of a mechanism whereby development is checked as well as one whereby it is initiated.

The following is a preliminary report of a series of investigations performed in an attempt to demonstrate the actual existence of such a mechanism.

Inhibitory Dominance.—Rand and Browne (1926) from a number of grafting experiments and Rand and Ellis (1926) from their work with individuals of Planaria maculata which had been made double-headed by a longitudinal splitting in the mid-line concluded that removal of one of the two heads may be followed by its regeneration at a retarded rate, or by non-regeneration in which event the animal resumes the form of a typical single-headed worm. The non-regeneration was said to be due to an "inhibitory dominance" exerted by the remaining head.

The above experiments dealing with double-headed planarians arising from the splitting operation have been extended and modified so that data from 225 cases involving a wide range of duplex forms, some with equal and others with unequal heads, are now available. Careful measurements of the duplicated regions and of the common posterior part were made, and the level of transection carefully noted.

It has been definitely demonstrated that removal of one of the heads from a double-headed individual of Planaria maculata may result in the regeneration of an aborted head, in regeneration at a retarded rate or in no regeneration (Goldsmith, 1930a). The failure to regenerate is not due to the lack of regenerative capacity, nor can it be viewed as being due to the absence of the nervous or digestive systems. This failure is correlated with the presence of the remaining head. The effectiveness of this head as an inhibitor is in turn correlated with: (1) its size in relation to the size of the removed head; (2) length of stump, i.e., the length of the duplicated region following head removal; (3) body-level, i.e., level on the antero-posterior axis at which the incision for removal of the supernumerary head is made.

It was found that the frequency of head regeneration in the double-

headed worms was greatest in a zone lying between a level somewhat posterior to the eyes and a level anterior to the pharynx.

Induction.—Child (1927) produced secondary hydranths in Corymorpha by a series of incisions radiating from a common center. Utilizing a similar technique (Goldsmith, 1930b), and also those of faradic stimulation and cautery (Goldsmith, 1933), it was found possible to induce supernumerary outgrowths which ranged in form from mere small bulges or indifferent conical outgrowths to head-like structures possessing typical eyes. In a number of cases supernumerary eyes resulted from the operation.

The results derived from the three types of injury to individuals of Planaria maculata agree in that the structures developing after stimulation in the pre-pharyngeal region differentiate to a greater extent than do those arising after stimulation in the post-pharyngeal region.

Cautery experiments with Procotyla gave negative results.

Discussion.—Santos (1929, 1931), using Planaria maculata and Planaria dorotocephala, performed a series of homoplastic and heteroplastic transplants of the region of the cephalic ganglia. In its new location *the graft developed into a head-like structure and then induced an outgrowth from the host body* (part of the outgrowth appeared to arise from donor tissue). Although the supernumerary structures produced by non-specific stimulation closely resemble those produced by grafting cephalic ganglia the sequence of events is quite different. In the former the injured region *becomes elevated.* This *elevation increases in size* so that it protrudes markedly from the host, and *eyes and auricles may develop in the distal portion of the structure so formed.* This behavior and that of the Corymorpha individuals described by Child would then necessitate shifting the emphasis from the agent utilized (injury or implantation of tissue) to the zone to which the agent is applied or transplanted.

From two contrasting types of experimentation, it has been made apparent that a zone lying between a level somewhat posterior to the eyes and one somewhat anterior to the pharynx is of special significance in that: (1) the frequency of regeneration of one of the heads of a double-headed form is greater, and (2) structures arising after non-specific stimulation differentiate to a greater extent in this zone.

Further, these results are in agreement with recent experimental cytological and chemical findings.

Curtis and Schulze (1934) have concluded that certain cells—formative cells—are present in the parenchyma of planarians. They maintain that these cells have the capacity to give rise to all new tissues and that their number is greater in members of species with a high regenerative potency (Planaria maculata) than it is in worms with a low regenerative potency

(Procotyla fluviatilis). They further state that the number of formative cells is greatest in a zone lying somewhat posterior to the eyes and somewhat anterior to the pharynx.

Buchanan (1933) criticizes the view that formative cells are to be correlated with regeneration. He states in part (p. 197) ". . .the inhibitory effect on head formation is much greater if the level of origin of the piece is shifted posteriorly than if the size of the piece is reduced. Presumably, in reducing the size of the fraction from one-half to one-eighth the total length of the animal, one reduces the number of neoblasts and the quantity of nerve tissue correspondingly; yet the reduction in head frequency is small as compared with the effect of shifting the level of origin of the piece."

It appears to me that the important factor is the number of formative cells per unit volume, and this number is smaller at a more posterior level. By reducing the size of an anterior piece *one does not reduce the density of neoblasts;* on the contrary since injury may cause these cells to divide (Flexner, 1898; Curtis, 1902) the number would be made even higher.

Coldwater (1930) has reported that the glutathione content in Procotyla is low while that in Planaria agilis is high; further, that the glutathione content is higher in regenerating tissues. These results Coldwater correlated with the formative cell content. Curtis (1928, 1930) has shown that the formative cells are destroyed by x-ray. Exposure of glutathione to radium emanation decreases the free SH (Hammett, 1932).

Hammett, from an extensive series of experiments employing a variety of plant and animal forms, claims that the sulfhydryl group acts as an accelerator of cell division which in turn "enhances differentiation," and that the partially oxidized derivatives retard cell multiplication.

In view of the previous investigations in part here cited and of the experimental results briefly presented it is suggested that the high degree of differentiation attained by the outgrowths induced by the cautery operation and the ineffectiveness of the inhibitory mechanism in the pre-pharyngeal zone (above referred to) are correlated with a high formative cell number and a high SH content in that region. It is possible that the inhibitory action may be exercised by means of a partially oxidized derivative of SH. It must be borne in mind that sulfoxide arising as a product of the partial oxidation of the SH may be formed in the region of injury and also in small amounts throughout the body. However, it may be urged that these quantities are not of themselves capable of exerting a restraining influence sufficiently powerful to prevent regeneration. If the above interpretation be correct then the difference in behavior in the regeneration of a head in a single-headed animal and that in a double-headed animal would be due to the additional sulfoxide arising in the region somewhat posterior to the eyes.

Finally, it is suggested that regeneration in Planaria maculata is not to be accounted for in terms of "head stuffs" and "tail stuffs." Rather it is to be regarded as due to the innate property of the formative cells to respond to an injury by proliferating and differentiating to the utmost—in planarians the height of differentiation is expressed in head formation. The degree of differentiation which the regenerate undergoes depends upon the ratio of the regenerative potency present in the region of injury to the inhibitory action exerted by the apical end of the worm.

[1] Acknowledgments are due to the Department of Biology of New York University (Washington Square College) for assistance given the author during 1929–31.

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DOES "PROTECTIVE COLORATION" PROTECT?—RESULTS OF SOME EXPERIMENTS WITH FISHES AND BIRDS

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Those who have taken part in the interminable controversy regarding the protective value of animal coloration have seldom concerned themselves with the phenomena of color change, displayed so strikingly by the lower vertebrates and some other groups. Yet here, one would expect, the most important evidence on the subject should be found. It is hardly conceivable that there should have been evolved such a widely distributed and highly complex mechanism, involving sensory, neural and effector elements, unless it were in some way vitally important to the animals in question. And so far as fishes at least are concerned, only one interpretation of this function has been proposed which seems to have the least plausibility. This is the obvious one that we have to do with an adaptation for concealment, either from enemies or from prospective prey. The only other type of explanation, so far as I know, which has been applied to the chromatic function in animals appeals to possible advantages derived from the absorption or reflection by the body surface of radiant energy, particularly that of the infra-red region. This hypothesis was proposed years ago by Max Weber, in relation to certain amphibia, and indeed it may have a limited application to some of the land-living vertebrates. But as applied to aquatic animals, particularly such as remain perpetually submerged, it would seem to be inconsistent with the most elementary data of physics. Moreover, it would not explain those changes—the most familiar ones of all—which bring the organism into harmony with its background. And least of all would it explain changes in the distribution of light and shade in the skin of some of the flatfishes—in the animals' pigment *pattern*—which is now known to be surprisingly responsive, at times, to the texture of the sand or gravel on which they lie, or to artificial patterns on the bottoms of the aquaria.

However, no argument for the efficacy of "protective coloration," fixed or changeable, is likely to be convincing so long as we merely rest our case on the assertion that no other explanation is possible. The author has planned for years to undertake an experimental study of this question, but only recently has he had the opportunity to carry out such experiments. The general procedure to be followed seems so obvious that the wonder is that no such experiments have been conducted in the past. Fishes, well-adjusted and ill-adjusted to a particular background, are placed in equal numbers in tanks of the shade in question and exposed to the attacks of

fish-eating birds. The proportionate numbers of individuals of the two shades which are eaten in a given time are recorded.

Not only does the procedure for such an experiment seem to be obvious, but the outcome might appear to be predictable with complete confidence. However, with Fuchs,[1] Verne[2] and others denouncing the entire protective coloration idea as "finalistic," "teleological," "anthropomorphic," etc., and with McAtee asserting a wholly non-selective choice of food-animals by birds,[3] it is plain that one can no longer base one's arguments on inherent probability alone. Detailed evidence seems to be called for.

The following pages record the results of my first series of experiments in this field. My entire program is by no means completed. Arrangements have been made for employing other birds, probably waders, and perhaps predators belonging to other classes of vertebrates. Since, however, the work cannot be resumed for another month or two, and since the first series of experiments, conducted with penguins, have yielded altogether decisive results, it seems worth while to report my observations to date.

It gives me great pleasure to acknowledge here the assistance of members of the staff of the San Diego Zoölogical Gardens, and in particular of the director, Mrs. Belle J. Benchley, without which these studies would have been altogether impossible. It has been my privilege to carry out this entire work at our San Diego "Zoo," and to use birds from its collections for the purposes of my experiments.

My acknowledgment is also due to Superintendent Alvin Seale and Mr. W. R. Martin of the Steinhart Aquarium, San Francisco, for providing the considerable number of *Gambusia* here employed.

In preparation for the experiments, "mosquito-fishes" (*Gambusia patruelis* (Baird and Girard)) were placed in large numbers in two cement tanks, painted black and white, respectively. They were kept in these tanks for from seven to eight weeks before being withdrawn for use. As is well known, many fishes undergo rather rapid changes of shade when transferred from a light to a dark background or the reverse. It is now known that in addition to these prompt changes of shade or color, due to the shifting of the pigment within the chromatophores, more radical changes occur, resulting from an actual increase or loss of pigment or of entire chromatophores.

Thus, fishes kept for long periods upon black, white or other backgrounds, reach degrees of pigmentation which are not promptly modified when the animals are transferred to new and different backgrounds. A fish which has been long habituated to black pales considerably within a few minutes, it is true, when it is transferred to white, but it remains for hours, sometimes for days, distinguishable from a fish which has been kept for a long time on white. The converse is true of a fish which is transferred to black after a long sojourn upon white.

The "black" fishes used in the present experiments, when freshly taken from the black aquarium, were nearly dead black upon the dorsal and dorso-lateral surfaces. The "white" fishes, on the other hand, appeared pale buff or gray, rather than white, when viewed against a white background, though appearing nearly white when seen against black. The rate at which these appearances change, after transfer to white or black containers, respectively, is being studied photographically, and will be discussed in my final report upon these experiments. A very noticeable change occurs within a few minutes.

As predators, I have employed successfully only the Galapagos penguin (*Spheniscus mendiculus Sundevall*). This bird was chosen because of its keen appetite for fishes, and its complete indifference to the presence of observers. A cormorant was tried on one occasion, but proved to be altogether too wild for the purpose. It did not swallow a single fish, though it killed five when first placed in the black tank.[4] The penguins were put into the tanks two at a time. In most cases they had been fed on the preceding day; in one instance, the interval since feeding was two days. Since the birds were taken at random from a stock of about a dozen, it is likely that more than two individuals were represented in the experiments.

The tanks used were wooden ones, specially constructed for the purpose. Their internal dimensions were eight by fifteen feet, with a depth of three feet. They were filled with fresh water to a depth of two to two and a half feet. One was painted dead black within, the other a very pale gray, lying between Ridgway's "pale olive-gray" and "light olive-gray." The bottoms were kept fairly free from leaves or sediment which might obscure them.

It is evident that in the present series of experiments, we are not dealing with differences in color, *sensu stricto*, but merely with differences in shade. Thus, the problem of the extent to which birds possess color-vision has not been touched upon.

In each experiment, an equal number of "black" and "white" fishes kept until that time in jars of the shade to which they had been habituated, were emptied together into one end of the tank. The birds were dropped in at the other end, either simultaneously with the fishes, or after a definite interval. They sometimes began the pursuit of the fishes immediately, sometimes after the lapse of a half minute or a minute. The time of sojourn of the birds was recorded from the moment when they began to chase the fishes.

The fishes, when first placed in the tank, tended to form a single school, regardless of color. So far as noted, the "whites" and "blacks" behaved exactly alike. After the onslaught of the birds, however, this school was broken up, the fishes scattering in all directions, and tending to concentrate in the corners. Neither the birds nor the fishes were disturbed by us

in any way, during the feeding-test, except that on a few occasions, the fishes were driven out of a corner by a stick.

The penguins, much of the time, swam under water, though coming to the surface at frequent intervals for air. In the pale tank, they could be readily seen pursuing the fishes; in the dark tank this was less easy to see.

After leaving the birds in the tank for a period which was believed sufficient to enable them to catch about half of the fishes, they were removed. The surviving fishes were next removed. A movable partition of wire screen was pushed from one end of the tank to the other, and the fishes thus penned in were caught with a rectangular hand-net. Great care was taken to remove all of them.[5]

RESULTS OF EXPERIMENTS UPON PROTECTIVE VALUE OF CONCEALING COLORATION IN FISHES (*Gambusia* DEVOURED BY PENGUINS)

DATE 1934	NUMBER OF EXPERIMENT	TANK	NUMBER FISHES	INTERVAL BETWEEN ADMITTING FISHES AND BIRDS	TIME ALLOWED BIRDS IN TANK	SURVIVORS BLACK	SURVIVORS WHITE	SURVIVORS DOUBTFUL	EATEN BLACK	EATEN WHITE	EATEN DOUBTFUL
Aug. 3	1	Pale	100	None	2 min. +	36	44	0	14	6	0
	2	Pale	100	None	4′10″	21	36	0	29	14	0
	3	Pale	76	None	4 min.	16	19	0	22	19	0
Aug. 9	8	Pale	300	11 min.	6 min. +	48	84	2	100	64	2
Totals			576			121	183	2	165	103	2
									61%	38%	1%
Aug. 6	4	Black	90	None	4 min.	32	11	0	13	34	0
	5	Black	90	None	5 min. +	28	2	0	17	43	0
	6	Black	90	5 min.	5 min.	28	3	0	17	42	0
Aug. 9	7	Black	200	10 min.	8–9 min.	74	18	0	26	82	0
Totals			470			162	34	0	73	201	0
									27%	73%	

The identification of the fishes recovered, as "blacks" and "whites," was not difficult, despite the considerable degree of convergence of the two lots which took place when placed together on a common background. In the few cases in which certain identification was not immediately possible, recourse was had to treatment with a one per cent solution of urethane. This brings about an identical tonus of the chromatophores in the two lots, thus revealing the marked differences in the amounts of melanin present. The two specimens marked "unidentifiable" in the table were killed by the birds, but not eaten.

The results of these eight tests are presented in the accompanying table. It will be seen that 1046 fishes were employed in all, of which 544 or somewhat more than half, were eaten by the birds. Very nearly equal numbers were eaten in the pale and black tanks (270 and 274, respectively).

The most important figures are those giving the percentages of "black" and "white" fishes eaten in each tank. *Of those consumed in the pale tank,* 61 *per cent were "blacks,"* 38 *per cent were "whites" and* 1 *per cent unidentifiable. Of those consumed in the black tank, on the other hand,* 27 *per cent were "blacks" and* 73 *per cent were "whites."* Moreover, the single experiments for each tank all show the same trend. There are no exceptional cases.

It will be noted that in three cases the fishes were left in the tanks for periods of five to eleven minutes before the birds were introduced. This was for the purpose of allowing a certain amount of change to occur in one or the other of the two lots of fishes, thus diminishing the difference between the two. In reality, no significant effect resulted in the ratios between the "adapted" and "non-adapted" fishes eaten. In the case where the fishes were left in the pale tank for eleven minutes before admission of the birds, the ratio is almost exactly the same as the mean figure for the other tests. The same is true, for the third test in the black tent, when a five-minute interval was allowed. Rather curiously, in the case where a ten-minute interval was allowed in this tank, the proportion of "whites" eaten was slightly greater than the average (76 per cent). It can hardly be doubted, however, that had considerably longer periods been allowed in each case for the less adapted fishes to adjust themselves to their new background, the capture of fishes by the birds would have become more and more indiscriminate. Theoretically, an interesting series of experiments would be possible here, in which longer and longer intervals were allowed for the fishes to approximate one another in shade. But it would be important to employ in each case the same individual birds, *in the same state of hunger.*

In the course of these experiments, the question naturally arose whether this differential mortality of the fishes, under the attacks of the penguins, may not have been due, in part at least, to differences in the activity of the fishes themselves. It had long been noted in the laboratory, both for *Lebistes* and *Gambusia*, that specimens kept in white aquaria were far more shy than those kept in black ones. The former retreated much more rapidly than the latter when, for example, the hand of the observer was waved over the container. Special experiments showed that "blacks," after transfer to a white aquarium, gradually approximated the "whites" in shyness and activity; likewise that "whites," in a black aquarium, rather rapidly calmed down to a state in which they were nearly as quiet as fishes of continuously "black" history. In neither case, however, did the transposed fishes attain the degree of activity (or quiescence) shown by those which had been long habituated to a given background, at least within a period of forty minutes.

So far as these results may bear upon the bird-feeding experiments, they

would seem to make it probable that "black" fishes, in the pale tank, would be subject to an additional handicap, besides their greater conspicuousness. They would probably show a somewhat diminished propensity to escape from a pursuer.

On the other hand, "white" fishes, in the black tank, would probably be somewhat more adroit in escaping than the "blacks," a circumstance which throws into greater relief the handicap which they undoubtedly suffer by reason of their greater conspicuousness. We are not, of course, in a position to evaluate the relative importance of visibility and motility in preserving these fishes from capture by birds which are fully capable of overtaking them in the water. In the feeding experiments herein discussed, it is significant that the most striking results were obtained in the black tank, in which the handicap of greater visibility, on the part of the "whites," might have been supposed to be partially offset by a greater motility.

It seems evident, in view of all these facts, that fishes which harmonize in shade with their immediate surroundings are less likely to be eaten by birds (or at least by certain birds) than fishes of the same species which do not so harmonize. If this is true, we can hardly doubt that the capacity of fishes to adjust their appearance to their background is highly useful to them. To what extent slight differences in this capacity would be of survival value is a question upon which we have no evidence. But it seems quite probable that, considered statistically, even scarcely perceptible differences of this sort would be sufficient to affect the mortality rates, were we concerned with sufficiently large populations. In any case, any one who has experimented in this field knows that not only different species of fishes, but also different individuals of the same species, show marked differences in the perfection of their mechanism for color change. We thus have some of the essential conditions for the evolution of this mechanism through natural selection.

[1] Fuchs, R. F., *Sitzungsber. der physikalisch-mediz. Soc. in Erlangen*, Bd. **44**, S. 134–177 (1913); Winterstein's *Handbuch der vergleichenden Physiologie*, Bd. **3**, Hälfte 1, Teil 2, S. 1189–1656 (1914).

[2] Verne, J., *Les pigments dans l'organisme animal.* Paris: Gaston Doin et Cie, 603 pp. (1926).

[3] "Availability undoubtedly is the chief factor involved in the choice of food, and predation therefore tends to be in proportion to population.... Considering bird predation alone this principle leads to a high degree of indiscriminacy in attack upon the whole kingdom of animal life.... *And this is only another way of saying that the phenomena classed by theorists as protective adaptations have little or no effectiveness*" (italics mine). McAtee, W. L., Smithsonian Miscellaneous Collections, **85**, No. 7, 1–201 (1932).

[4] It is perhaps worth recording, in view of the results with penguins, that four of the five fishes captured by the cormorant in the black tank were "whites."

[5] I must acknowledge the important services of my son, Herbert Sumner, who spent some hours in the water of these tanks engaged in this not very thrilling type of fishing.